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Abstract	<p>The incidence of cardiovascular disease due to thrombosis is 2–4 folds greater in diabetic patients. Prothrombin time, activated partial thromboplastin time and platelet count are hematological indices that give an insight into the coagulation status. Hence, this study aims to assess the coagulation status of type II diabetic patients.</p> <p>A comparative cross-sectional study was conducted at Bahir Dar Felege Hiwot referral hospital, Northwest Ethiopia. A total of 40 treated type II diabetic, 40 untreated diabetics and 40 non-diabetic subjects were included. After taking informed consent, structured questionnaire was used to collect socio-demographic data. Following interview, 4 ml of blood was collected to determine PT, aPTT and platelet count of the three groups. The data were entered into SPSS version 20 and analyzed. One-way ANOVA was used to compare means of PT, aPTT and platelet count among the groups. A <i>P</i> value less than 0.05 was considered as statistically significant.</p> <p>The mean aPTT of non-diabetic, treated and untreated type II diabetic patient was <math>32.8 \pm 4.12</math> s, <math>34.4 \pm 5.3</math> s, and <math>25.42 \pm 8.46</math> s, respectively. The proportion of untreated diabetic patients with normal PT, aPTT and platelet counts was 60.0%, 7.5 and 92.5%, respectively. There was a significant shortening of aPTT in untreated diabetic as compared to both treated and non-diabetic controls (<math>P &lt; 0.001</math>).</p> <p>Shortening of aPTT in untreated type II diabetic patients might be useful marker in patients with diabetes. Therefore, monitoring the aPTT in newly diagnosed diabetic patients is important.</p>	
Keywords (separated by '-')	Activated partial thromboplastin time - Platelet count - Prothrombin time - Type II diabetes mellitus - Bahir Dar - Ethiopia	
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## RESEARCH ARTICLE

# Prothrombin time, activated partial thromboplastin time and platelet counts of type II diabetes mellitus: a comparative study

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## Abstract

**Background** The incidence of cardiovascular disease due to thrombosis is 2–4 folds greater in diabetic patients. Prothrombin time, activated partial thromboplastin time and platelet count are hematological indices that give an insight into the coagulation status. Hence, this study aims to assess the coagulation status of type II diabetic patients.

**Methods** A comparative cross-sectional study was conducted at Bahir Dar Felege Hiwot referral hospital, Northwest Ethiopia. A total of 40 treated type II diabetic, 40 untreated diabetics and 40 non-diabetic subjects were included. After taking informed consent, structured questionnaire was used to collect socio-demographic data. Following interview, 4 ml of blood was collected to determine PT, aPTT and platelet count of the three groups. The data were entered into SPSS version 20 and analyzed. One-way ANOVA was used to compare means of PT, aPTT and platelet count among the groups. A *P* value less than 0.05 was considered as statistically significant.

**Results** The mean aPTT of non-diabetic, treated and untreated type II diabetic patient was  $32.8 \pm 4.12$  s,  $34.4 \pm 5.3$  s, and  $25.42 \pm 8.46$  s, respectively. The proportion of untreated diabetic patients with normal PT, aPTT and platelet counts was 60.0%, 7.5 and 92.5%, respectively. There was a significant shortening of aPTT in untreated diabetic as compared to both treated and non-diabetic controls ( $P < 0.001$ ).

**Conclusions** Shortening of aPTT in untreated type II diabetic patients might be useful marker in patients with diabetes. Therefore, monitoring the aPTT in newly diagnosed diabetic patients is important.

**Keywords** Activated partial thromboplastin time · Platelet count · Prothrombin time · Type II diabetes mellitus · Bahir Dar · Ethiopia

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## Background

Diabetes mellitus (DM) is metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Etiologically, diabetes is classified into two; type I and type II diabetes mellitus [1]. Type II diabetes mellitus, previously called non-insulin dependent diabetes mellitus, is characterized by decreased insulin sensitivity which can subsequently provoke decreased insulin secretion as a result of Beta-cell loss [2].

The International Diabetes Federation (IDF) has predicted that the number of individuals with diabetes will increase from 382 million in 2013 to 592 million in 2035, with 77% of the disease burden in lowland middle-income countries. Similarly, the IDF have also reported that in Ethiopia about 2 million people are expected to live with DM with the national prevalence of 4.4% in 2013 [3, 4]. Type II diabetic patients are at high risk for the development of thrombosis and bleeding disorders. Approximately 80% of patients die as a result of cardiovascular

complications and its incidence due to thrombosis is 2–4 folds greater than the general population [5].

Furthermore, prothrombin time (PT), activated partial thromboplastin time (aPTT) and platelet are hematological indices that give an insight into the coagulation status of patients. PT is a screening test used to detect disorders involving the activity of clotting factors (proteins) such as I, II, V, VII, and X of the extrinsic and common pathways while aPTT is used to screen for abnormalities of the intrinsic and common clotting systems and to monitor the anticoagulant effect of circulating heparin. It measures the activities of factors I, II, V, VIII, IX, X, XI, and XII of the intrinsic and common pathways [6]. However, there was no previous study that had been conducted concerning on evaluating the coagulation status of diabetes type II patients in the study area. Therefore, this study was aimed to assess the prothrombin time, activated partial thromboplastin time and platelet counts of type II diabetes mellitus at Bahir Dar Felege Hiwot referral hospital, Northwest Ethiopia.

## Methods

### Study setting and study population

A comparative cross-sectional study design was used to determine and compare PT, aPTT and PLT count of treated type II DM, untreated DM and healthy individuals. The study was conducted at Bahir Dar Felege Hiwot referral hospital from March to April, 2015. This hospital is found in Bahir Dar town which is 546 km from Addis Ababa in the Northwest Ethiopia. The hospital provides services for more than 800 new out patients and 5–10 follow-up diabetes patients per day.

According to the rules of thumb that has been recommended by VanVoorhis and Morgan, sample size of 30 for 80% power in each group are required to detect real differences [7]. Based on this suggestion, a total of 120 conveniently selected study subjects (40 treated type II DM patients, 40 untreated DM patients and 40 healthy controls) aged 30–60 years were included in this study. The study subjects were categorized in to three groups. Group I consisted of type II diabetic patients who had already started their non-insulin hypoglycemic drugs while group II included untreated newly diagnosis diabetic patients (fasting blood sugar (FBS) > 126 mg/dl or random blood sugar (RBS) > 180 mg/dl, had symptoms of diabetic and were confirmed by physician). On the other hand group II included those individuals who were apparently healthy and came to the hospital for medical check-up (Individuals who did not have a diabetic history or symptom, any anticoagulant therapy, hypertensive, clinically proven liver dysfunction and whose FBS level was between 70 mg/dl and 110 mg/dl) were studied. Treated type II diabetic and newly diagnosis DM patients on warfarin or heparin or any other anticoagulation therapy such as aspirin, and with

other complications such as history of liver diseases, liver dysfunction, on hepatotoxic drugs, history of alcohol intake or cigarette smoking, hypertensive and psychic patients were excluded from the study. Apparently healthy individuals with any of DM symptom or who had taken any anticoagulant drug or who have a history of hypertensive were also excluded from the study.

### Data collection and laboratory analysis

Sociodemographic data were collected with face to face interview after taking informed written consent followed by 4 ml of fasting blood collection. Then 1.8 ml blood sample was delivered into a test tube containing 0.2 ml tri-sodium citrate anticoagulant to keep 9:1 ratio of blood to anticoagulant. The blood was then centrifuged at approximately 3000 rpm for 15 min to prepare platelet poor plasma for aPTT and PT analysis. aPTT and PT were analyzed by using Huma Clot Duo<sup>plus</sup> instrument (HUMAN GmbH, Germany) [8]. The remaining blood was delivered into EDTA test tube for platelet count using Huma-Count hematology analyzer (HUMAN GmbH, Germany) [9].

To assure the quality of laboratory tests; proper labeling, patient identification and sample collection procedures were strictly followed. Quality control samples were also analyzed along the study subject samples. Appropriate recording, results interpretation and cross checking the result with its correspondence labeling were performed. Completeness of data was checked daily.

### Statistical analysis

Data were entered in to SPSS version 20 for analysis. Descriptive statistics were used to describe the study groups. The Kolmogorov-Smirnov normality test was conducted and it showed that continuous variables were normally distributed among each group, so parametric tests were used. All continuous variables results were presented as mean ± standard deviation (SD) for each group. One-way ANOVA was used to compare means of PT, aPTT and platelet count among the groups. Tables and graphs were used to present the results. A  $P < 0.05$  was considered as statistically significant.

**Data availability** All data supporting these findings is contained within the manuscript.

## Results

### Characteristics of study participants

A total of 120 study participants were included in this study. Of which, 40 were untreated DM patients, 40 were treated type II DM patients and the remaining 40 were healthy

**Table 1** Demographic data of treated, untreated diabetic and non-diabetic study subjects

Parameters	Untreated DM N (%)	Treated type II DM N (%)	Healthy controls N (%)
<b>Gender</b>			
Male	25 (62.5%)	25 (62.5%)	24 (60.0%)
Female	15 (37.5%)	15 (37.5%)	16 (40.0%)
<b>Age in years</b>			
30–40	23 (57.5%)	30 (75.0%)	26 (65.0%)
41–50	12 (30%)	9 (22.5%)	13 (32.5%)
51–60	5 (12.5%)	1 (2.5%)	1 (2.5%)
<b>Residence</b>			
Urban	23 (57.5%)	28 (70.0%)	29 (72.5%)
Rural	17 (42.5%)	12 (30.0%)	11 (27.5%)
<b>Marital status</b>			
Married	25 (62.5%)	30 (75.0%)	25 (62.5%)
Not married	15 (37.5%)	10 (25.0%)	15 (37.5%)
<b>Educational status</b>			
Illiterate	12 (30.0%)	17 (42.5%)	8 (20.0%)
Elementary school	6 (15.0%)	6 (15.0%)	7 (17.5%)
High school	11 (27.5%)	9 (22.5%)	10 (25.0%)
University graduated	11 (27.5%)	8 (20.0%)	15 (37.5%)
<b>Duration of treatment</b>			
0–5 years	NA	28 (70.0%)	NA
6–10 years	NA	10 (25.0%)	NA
> 10 years	NA	2 (5.0%)	NA

N.B: DM, diabetic miletus; NA, not applicable

controls. The minimum and maximum age was 30 and 56 with the mean age of  $40.57 \pm 7.47$  years in untreated,  $37.80 \pm 5.9$  years in treated and  $39.27 \pm 6.59$  years in healthy controls. About 62.5%, 75 and 62.5% of untreated DM, treated type II DM and non-diabetic controls were married, respectively. Furthermore, 70% of treated type II DM patients were treated using non-insulin hypoglycemic drug for less than 5 years and only 2% of treated type II DM were treated for more than 10 years with similar drug (Table 1).

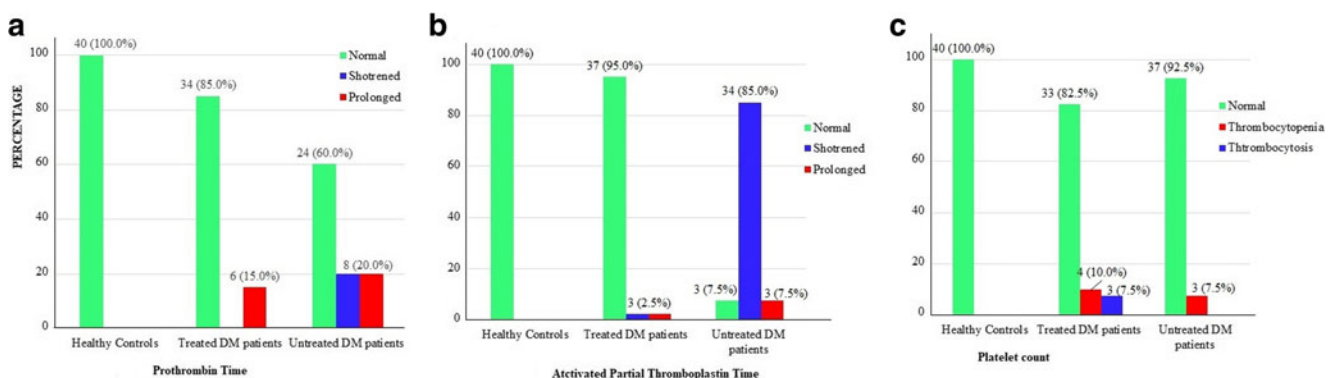
## Laboratory findings

The proportion of untreated diabetic patients with normal PT, aPTT and platelet counts was 60.0%, 7.5 and 92.5%, respectively. Whereas, the proportion of treated diabetic patients with normal PT, aPTT and platelet counts was 85%, 95%, and 82.5%, respectively. Moreover, the proportion of treated diabetic patients with decreased aPTT and platelet count was 2.5 and 7.5%, respectively. There was no decreased PT value in treated DM patients. Whereas, the proportion of untreated diabetic patients with decreased PT, aPTT and platelet count was 20%, 85 and 7.5% of respectively (Fig. 1a, b & c).

The mean aPTT of non-diabetic, treated and untreated type II diabetic patient was  $32.8 \pm 4.12$  s,  $34.4 \pm 5.3$  s, and  $25.42 \pm 8.46$  s, respectively. Similarly, the mean PT of non-diabetic controls, treated type II DM and untreated DM were  $14.28 \pm 1.50$  s,  $14.65 \pm 2.50$  s and  $13.54 \pm 3.44$  s, respectively. Moreover, the platelet count of non-diabetic control, treated type II DM, and untreated DM were  $251,000 \pm 71,964$ ,  $254,000 \pm 95,077$  and  $250,000 \pm 75,546$ , respectively. The study subjects had significantly different aPTT among the three groups in the ANOVA test ( $F: 60.7$ ;  $P < 0.001$ ). There was a significant shortening of aPTT in untreated diabetic as compared to both treated and non-diabetic controls ( $P < 0.001$ ). However, the mean aPTT, PT and platelet count were not significant between treated type II DM patients and non-diabetic individuals ( $P > 0.05$ ) (Table 2).

## Discussion

In this study, non-diabetic, treated and untreated diabetic have a mean aPTT of  $32.79 \pm 4.12$  s,  $34.45 \pm 5.35$  s, and  $25.42 \pm 8.46$  s, respectively. There was a significant shortening of aPTT in untreated diabetic patients compared to either treated or non-diabetic individuals ( $P < 0.001$ ). Similar findings were reported by Zhao et al... among patients in China [10], by Chavan et al. in India [11], Acang et al. in Indonesia [12] and Ankalayya et al in India [13] that described shortening of aPTT in type II DM patients is a high risk of hypercoagulable state.

**Fig. 1** Profile of PT, aPTT and platelet count in healthy controls, treated and untreated diabetic study subjects



**Table 2** Mean of PT, aPTT and platelet count of treated and untreated diabetic study subjects

Parameters	Untreated DM Mean $\pm$ SD	Treated type II DM Mean $\pm$ SD	P Value
PT/ Sec	13.54 $\pm$ 3.44	14.65 $\pm$ 2.50	0.274
aPTT/ Sec	25.42 $\pm$ 8.46	34.4 $\pm$ 5.35	<0.001
Platelet count/ $\mu$ l	250,000 $\pm$ 75,546	254,000 $\pm$ 95,077	0.759
Parameters	Untreated DM Mean $\pm$ SD	Non-diabetic Mean $\pm$ SD	P value
PT/ Sec	13.54 $\pm$ 3.44	14.28 $\pm$ 1.50	0.964
aPTT/ Sec	25.42 $\pm$ 8.46	32.79 $\pm$ 4.12	<0.001
Platelet count/ $\mu$ l	250,000 $\pm$ 75,546	251,000 $\pm$ 71,964	0.933
Parameters	Treated Type II DM Mean $\pm$ SD	Non-diabetic Mean $\pm$ SD	P value
PT/ Sec	14.65 $\pm$ 2.50	14.28 $\pm$ 1.50	0.405
aPTT/ Sec	34.4 $\pm$ 5.35	32.79 $\pm$ 4.12	0.951
Platelet count/ $\mu$ l	254,000 $\pm$ 95,077	251,000 $\pm$ 71,964	0.933

N.B: *P* value is derived from one way ANOVA test, numeric numbers in bold indicate the presence of significant association

This may happen due to the glycation of intrinsic clotting factors caused by the presence of persistent hyperglycemia in untreated DM patients. Persistent hyperglycemia may result the glycation of intracellular and extracellular protein that will change the normal functioning of these proteins which affect their clotting capacity [14]. Thus, glycation of clotting factors may result the activation of inactive intrinsic factors which finally results the shortening of aPTT [15].

However, PT of untreated DM patients was not found significantly associated in this study when compared to treated type II DM patients ( $P = 0.274$ ) and non-diabetic individuals ( $P = 0.759$ ). The insignificant PT results support the hypothesis that there is less involvement of the extrinsic pathway in hypercoagulability state in diabetic conditions due to the fact that injury occurring to the vascular system in diabetic patients does not involve the release of tissue factor from outside of the vascular system [16]. APTT but not PT would be affected by the glycation method. Hypercoagulability detected by shortened APTT values was independently associated with venous thromboembolism (VTE) and hypothesized that shortened APTT could be considered as a risk marker for VTE [17]. This entails that APTT is a better predictor of hypercoagulable state than PT in T2DM patients [18] which have any impact on the management of these patients.

The current study also showed that there was no significant difference of the mean PT, aPTT and platelet count between treated and non-diabetic individuals ( $P > 0.05$ ). This might be due to the effect of non- insulin hypoglycemic drug on glucose level which in turn prevents glycation process in treated DM patients.

In this study, the mean PT and platelet count of treated; untreated diabetic patients and non-diabetic individuals had

shown no significant difference among the groups ( $P > 0.05$ ). Similarly, studies conducted in Iran by Soltani et al. [16] and in Nigeria by Ifeanyi et al. [19] reported no significant difference of PT findings among the groups, and Ephraim et al. [20] also reported mean platelet count was within the normal range among study subjects in Ghana.

Moreover, 12.5% of untreated diabetic had a decreased PT compared to no decreased PT in treated diabetic patients. About 85% of untreated diabetic had a decreased aPTT whereas only 2.5% of treated diabetic had a decreased aPTT. This finding indicates that those treated type II diabetic patients had properly control their blood glucose level than untreated individuals. This may be due to association of glycation, a non-enzymatic binding of glucose on protein, with the persistence hyper glycemia that could change the normal function of the intrinsic factors (aPTT) and the extrinsic factors (PT) [14].

## Conclusions

There was a significant shortening of aPTT in untreated diabetic patients as compared with treated and non-diabetic groups. Therefore, shortening of aPTT in untreated type II diabetic patients might be useful marker in patients with diabetes. Monitoring of the aPTT in newly diagnosed diabetic patients is important to prevent hypercoagulation.

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**Authors' contributions** YAA participated in the design of the study, data collection, performed the statistical analysis and drafted the manuscript. MBS, MA and BE analyze and interpreted the data, and wrote the manuscript. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Ethical consideration** This study was reviewed and approved by the School of Biomedical and Laboratory Sciences Research and Ethical Committee, College of Medicine and Health Sciences, University of Gondar. Then, after official permission was obtained from Bahir Dar Felege Hiwot referral hospital, written informed consent was obtained from every individual prior to enrolment in the study. The purpose and objectives of the study was clearly explained to the study subjects. The respondent was allowed to quit if he/she didn't want to participate in the study. Confidentiality was maintained and withdraw at any time was allowed.

**Competing interest** The authors declare that there is no conflict of interests regarding the publication of this manuscript.

**Consent for publication** Not applicable. This manuscript does not contain any individual persons' data.

**Abbreviations** *aPTT*, activated partial thromboplastin time; *CVD*, cardiovascular disease; *DM*, diabetes mellitus; *FBS*, fasting blood sugar; *IDF*, international diabetic federation; *PT*, prothrombin time; *RBS*, random blood sugar

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